December 12, 1955

Dear Dr. Heumann:

I am very grateful to you for sending your manuscript on "Der Sexual zyclus sternbildender Bakterien", which I read with great pleasure. Your exact account of the cytological development of the star forms deserves congratulation, and, as you point out immediately provokes questions as to the design of experiments for genetical corroboration.

Actually, your suggestion that you visit our laboratory comes at a most propitious time, and I would be very happy to welcome you. I have been interested in the behavior of Agrobacterium tumefaciens and, with the help of Professor A. J. Riker have endeavoured to start a genetic program with this bacterium. However, though casual attention has been given to this program for the last several years, this species has proved to be an unusually difficult one in which the obtain auxotrophic mutants. I had an assistant who did a few preliminary experiments with other genetic markers, but with the pressure from her other duties she did not really have very much time for this study. We had speculated on the possibility of examining "Radio bacter" strains, but had done nothing about the idea. It happens also that our small laboratory is being rebuilt and enlarged so that we should have ample space to accomodate you.

Judging from your manuscript and my own experience, the combination of genetic and cytological methods should lead to the most fruitful results and I hope it will be possible for you to make the necessary arrangements to allow our collaboration in this laboratory. I would hope that you would be able to take leave for about a year's time in order to do this. We can accomodate you at any time after July 1, 1956 at which you can arrange to come. Please let me know to whom I should address any further letters in support of your proposal, Meanwhile, I would appreciate it if you could arrange for me to receive a brief curriculum vitae, and one or two letters of reference from your colleagues or professors, which may serve to help identify you for university purposes here.

There was only one point in your manuscript that was not clear to me: have you been able to follow the complete developmental cycle of a single star, or are your results based exclusively on a reconstruction of stages from mass cultures? If so, the type of technique which is outlined in the enclosed reprint may be of some use to your further studies.

In anticipation of your visit, it would be highly beneficial if we could both become better acquainted with each other's material. Under separate cover, I am sending you a bibliographic list and available publications of the genetic analysis of enteric bacteria; I would appreciate the same from you. In addition, would it be possible for you to send me sub-

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cultures of the strains with which you have been working, for the purpose of my own preliminary familiarization with your material. If the crossable strains of E. coli K-12 would be of any immediate interest to you, I would be happy to send them.

The experimental program that should be set up for your material is fairly obvious. First, genetic mutant "markers" (Merkmalen) will have to be obtained. As I indicated, A. tumefaciens gave only a few, very poor nutritional mutations, but the rhizobia might be better. If not, there are many other kinds of mutations, e.g., drug-resistance; motility; antigens, with which we have had considerable success with E. coli and with Salmonella, and which should be considered also. Actually, it should not be necessary to develop "selected" markers for your material-- since the presumptive sexual stage is morphologically defined, it should be necessary only to isolate zygotes (by micromanipulation) from mixed cultures and examine the unselected markers among the progeny. We had intended to do this with the not very sharp mutants we already had in tumefaciens, but your strains are obviously much more suitable material.

Fortunately I am able to contradict your remarks about the absence of any morphological correlation with sexual recombination in E. coli K-12. For about 18 months, I have been engaged in single-cell studies with highly fertile crosses (Hfr x F-) and the following picture has emerged. Recombination is evidently based on <u>conjugation</u> (as in Paramecium) rather than <u>copulation</u> (e.g., as proposed in the rhizobia). The two parental cells simply pair with one another, joined near their ends by a connection that has not yet been directly observed in living cells, but which is unmistakable by the association of the cells. The conjugal pairs last about an hour, then separate. Both cells remain viable and produce clones; only the clone from the F- parental cell includes recombinants. The entire process looks as if one (of several) nuclei is transmitted via a Verbindungsbrücke (cf. Potthof in Chromatium) from the Hfr to the (also multinucleate) F- cell, where a zygote nucleus results, followed immediately by segregation in what appear to be ordinary bacterial fissions. No cytological details that can be relied upon have been elucidated-- this will be one of my main concerns during the coming year, so you can see another reason why your visit will come at a most appropriate time.

You have also quoted the"conjugation tubes" of Bacillus megaterium, (deLamater) but I am most suspicious of them. Other workers have pointed out that the "tubes" may represent degenerated bacilli of a chain. As far as I know their development in living material has not been described. Finally, the confused claims of genetic recombination in this species have not been upheld by their own authors (e..g, Szybalski and Hunter).

Please let me know if I can be of any assistance to your plans, and keep me informed of their development.

Yours sincerely,

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